

## CONTROL OF WHEAT LEAF GROWTH UNDER SALINE CONDITIONS I. BIOPHYSICAL PARAMETERS OF LOCKHART GROWTH MODEL

Hamayun Arif, A. Deri Tomos\* & Nazir Ahmad

Department of Crop Physiology,  
University of Agriculture, Faisalabad

\*Ysgol Gwyddorau, Coleg y Brifysgol, Bangor,  
Gwynedd LL57 2 UW, Cymru, U.K.

The biophysical parameters controlling leaf growth were studied in context of salt stress. Various levels of NaCl, ranging from 25 to 250 mol m<sup>-3</sup>, were used to salinize the medium. The parameters were measured in growing zone of the first emerged leaf of wheat seedlings (cv. Flanders, a British variety). In case of leaf elongation rate a two phase response was observed, i.e. an immediate decrease followed by subsequent recovery. The elongation rate decreased within 1 to 2 minutes of the stress onset and later started recovering after 1 to 2 hours and was almost fully recovered after 24 hours for all the NaCl concentrations. Turgor pressure measured in epidermal and mesophyll cells was about 0.45 MPa in control plant cells. The turgor pressure did not change after the salt addition (0 to 150 mol m<sup>-3</sup> NaCl). However, it dropped when very high concentrations (200 and 250 mol m<sup>-3</sup>) of NaCl were applied to the medium. The salt stress did not have any effect on Instron tensiometric measurements (*in vitro*) of elastic and plastic extension of the cell wall.

### INTRODUCTION

Salinity affects plants at all stages of development particularly at the seedling stage prior to tillering (Rashid, 1986). The cause of leaf growth decline under such conditions is not fully understood. In this context several hypotheses have been put forward (Arif, 1990). All the suggested rationales are based on whole plant basis and most of the studies are carried out in long term (weeks and months) exposure of plants to the salt stress. For short term experiments, the measurement of growth of "linear" tissues can be conveniently characterised by the increase in tissue length (Pritchard, 1988). The growth inhibition could be due to osmotic or ionic components of salinity acting on biophysical and metabolic components of expansive growth

(Theil *et al.*, 1988). The biochemical mechanisms must always come down to the biophysical components to affect the growth (Tomos, 1988). The possible involvement of biophysical properties of cell wall in the control of growth has been proposed in a growth model (Lockhart, 1965).

$$r = \phi (P - Y) \quad (1)$$

where

- $r$  = rate of volumetric growth,
- $\phi$  = cell wall extensibility,
- $P$  = turgor pressure, and
- $Y$  = Yield threshold.

According to this formulation, growth rate is a function of a stress ( $P$ , the turgor pressure) and the rheological properties of

the cell wall ( $\phi$  and  $Y$ ). Involvement of cell wall properties such as rheology (Cleland, 1984; Greenway and Munns, 1983; Pritchard *et al.*, 1987) has already been postulated. The constancy of turgor pressure at different growth rates using tissue-averaged methods (Matsuda and Riazi, 1981; Michelena and Boyer, 1982; Matthews *et al.*, 1985) and using pressure probe (Pritchard *et al.*, 1987; Rich and Tomos, 1988; Thomas *et al.*, 1989) implies the involvement of  $\phi$  in control of growth. However, in saline conditions quite opposite has been observed as there is a decrease in turgor pressure but no change in  $\phi$  in bean leaves (Neumann *et al.*, 1988). It has, therefore, been planned to investigate the involvement of biophysical parameters in the control of leaf growth under saline conditions.

## MATERIALS AND METHODS

**Growth of plants:** Seeds of *Triticum aestivum* were soaked in aerated distilled water for 24 hours and germinated on 2.0 mm plastic mesh suspended over aerated half strength Hoagland solution (Hoagland and Arnon, 1950) contained in black painted plastic vessels (0.5 dm<sup>3</sup>). The germinated seedlings were kept in the dark for 2 days and then in continuous light for rest of the experimental period. After 7 days of germination different NaCl concentrations (0, 25, 50, 75, 100, 125 and 150 mol m<sup>-3</sup>) were added in one set to salinize the root medium, unless otherwise stated.

**Measurement of leaf elongation rate:** The elongation rate of the first emerged leaf of control and stressed plants was determined with a LVDT displacement transducer (Acevedo *et al.*, 1971; Arif, 1990).

**Measurement of turgor pressure:** Turgor pressure,  $P$ , was measured directly in individual epidermal and mesophyll cells of leaf growing zone using the pressure probe

(Hüsken *et al.*, 1978; Thomas *et al.*, 1989). The position of the growing zone in the leaf was determined using the technique of Kemp (1980). It was confirmed that the zone of maximum elongation was about 5 to 10 mm away from the base of the stem (shoot/root interface). The outer sheath was not removed completely to avoid the possible loss of turgor pressure by excessive evapotranspiration from the exposed surface. Instead, a window (2 x 2.5 mm) was cut carefully with a fresh scalpel blade through the outer sheath about 5 mm away from the stem base. For the measurements, the glass capillary of the pressure probe was passed through the window to reach to the cells.

**Measurement of cell wall extensibility:** Cell wall extensibility of the leaf growing zone was measured using the tensiometer (Instron) (Van Volkenburgh, 1983; Cleland, 1984; Pritchard *et al.*, 1988). Methanol killed tissues were subjected to two load cycles at a strain application rate of 2.3 mm min<sup>-1</sup>. The extensibility was calculated from the slopes of linear portions of the load-extension curves. Extensibility (both plastic and elastic) was expressed as percentage increase in the initial tissue length for a 15 g load.

## RESULTS AND DISCUSSION

In control conditions, the leaf was elongating at a rate of  $17.5 \pm 2.5 \mu\text{m min}^{-1}$ . On addition of salts, a decline in the elongation rate was observed in 1 to 2 minutes (Fig. 1) which continued decreasing for all the NaCl concentrations. In Figures 1, 2 and 3 the results of only two stress levels i.e. 25 and 150 mol m<sup>-3</sup> have been presented due to space limits. These results are in accordance with the responses first observed by Acevedo *et al.* (1971). Similar rapid responses have also been reported in response to the salt stress in barley leaves (Matsuda and Riazi, 1981; Thiel *et al.*, 1988).

**Table 1. Effect of NaCl stress on the (instron) cell wall extensibility (elastic and plastic)****a. Elastic extensibility (%/15 g load)**

NaCl concentration (MPa)	Time after stress (hours)		
	0	5	144
0	7.42 ± 0.49	7.62 ± 0.78	3.49 ± 1.70
25	7.08 ± 1.08	6.48 ± 0.90	2.82 ± 1.09
50	6.97 ± 0.88	6.76 ± 1.50	2.05 ± 0.64
75	6.41 ± 1.15	6.05 ± 2.36	2.04 ± 0.74
100	7.09 ± 0.98	6.27 ± 1.10	2.03 ± 0.85
125	6.86 ± 1.50	6.34 ± 1.09	2.13 ± 0.60
150	7.62 ± 0.99	6.72 ± 1.39	2.27 ± 0.70

**b. Plastic extensibility (%/15 g load)**

NaCl concentration (MPa)	Time after stress (hours)		
	0	5	144
0	3.82 ± 0.56	3.18 ± 1.22	1.23 ± 0.96
25	3.60 ± 2.60	2.20 ± 0.94	1.51 ± 0.95
50	3.10 ± 0.60	2.91 ± 0.60	0.59 ± 0.35
75	3.10 ± 1.68	2.13 ± 0.74	1.23 ± 1.14
100	2.61 ± 0.78	2.17 ± 0.91	0.65 ± 0.43
125	2.06 ± 0.81	2.15 ± 1.03	1.08 ± 0.52
150	3.34 ± 1.90	2.15 ± 1.03	0.91 ± 0.45

The elongation rate was curtailed more quickly for higher NaCl concentrations than the lower ones. When the salt was washed out from the root media, after 60 minutes of the stress, a big jump in the elongation rate was recorded instantaneously (within 1 to 2 minutes) resulting in very high growth rate; in most cases approximately the double of the control rates (Fig. 1). However, the increase was temporary and the elongation rate declined gradually in about 30 minutes to the normal values. It seems that the rapid recovery was not due to change in growing cell. Conversely, it might be due to

the stored growth as metabolic processes for cell expansion (Acevedo *et al.*, 1971) and/or to the cell division which might have continued unchecked during the stress period. In case of longer period (24 hours) treatments, a two phase response was observed in the elongation rate; an immediate decline following subsequent gradual recovery. After 2 to 3 hours of the media salination, a gradual and steady increase in the elongation rate was recorded which continued until the previous rate of elongation (prior to stress application) was attained (Fig. 2). This contrasts the observation of Thiel *et al.* (1988)

who reported the decreased but more stable elongation rate after about 1 hour of the stress initiation. The difference might merely be due to their shorter experimental period which had shown the complete recovery of leaf elongation rate if measured for longer period.

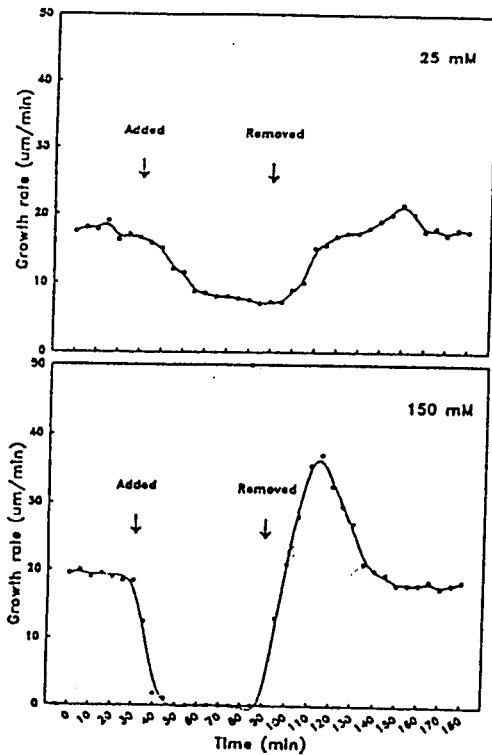


Fig. 1. Effect of NaCl on elongation rate of the first emerged leaf of 7 days old wheat seedlings (each point is the mean of 4-10 replicates taken from two experiments).

Taking into consideration the equation 1, when water conductivity to the expanding cells is not limiting, experiments were designed to see whether the drop in turgor pressure was responsible for the elongation rate decline. Turgor pressure measured in epidermal and mesophyll cells of leaf growing zone was on average  $0.45 \pm 0.08$  MPa for control plants. To our surprise, the tur-

gor pressure did not change in response to any of the added NaCl concentrations up to  $150 \text{ mol m}^{-3}$  (Fig. 3). even if it dropped, the change was much smaller than the level of detection by the technique employed. Thiel *et al.* (1988) found a drop in growing zone turgor pressure in response to salinity

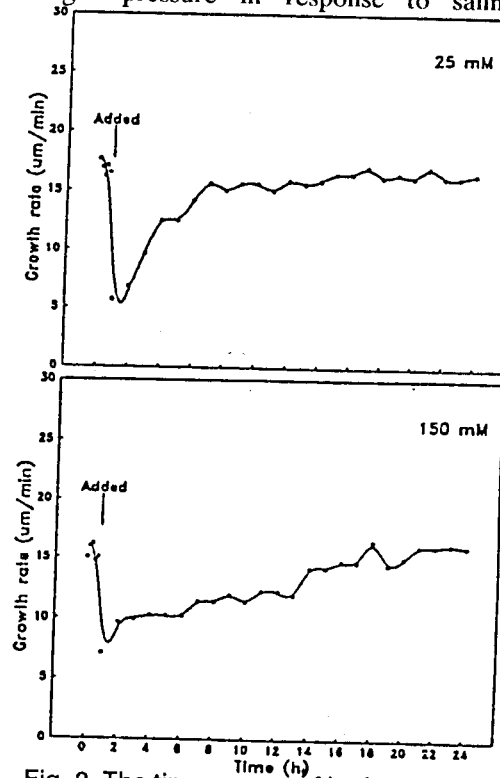


Fig. 2. The time course of leaf elongation rate recovery following the decrease in response to the NaCl stress (arrows indicate the time at which plants were introduced to the stress).

using pressure probe. They argue that the drop was restricted to the autotrophic leaves of the plants (third leaf in their case) not to the first leaf that utilises seed reserves for its growth. However, Thomas *et al.* (1989) could not detect such specificity even in the fourth leaf, exposed to low temperature stress. From our data it was observed that

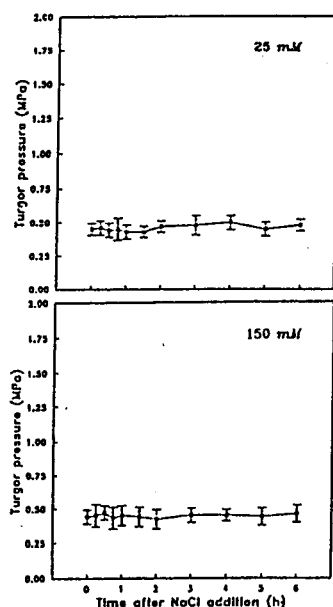


Fig. 3. Time course of turgor pressure in expanding cells of leaves in response to NaCl stress (each point is the mean of 5-25 replicates taken from three experiments and error bars indicate the standard deviation of the mean of all replicates).

either the turgor pressure was not responsible for the decline in elongation rate or the  $(P - Y)$  value is so small that it could not be detected. Hence, the observed decline in the elongation rate can be attributed to other biophysical parameters such as cell wall extensibility ( $\phi$ ) and yield threshold ( $Y$ ). Thomas *et al.* (1989) have also suggested the change in cell wall rheology as the cause of growth reduction at low temperature. Cosgrove and Sovonick-Dunford (1989) have proposed the growth retardation is due to a reduction in the  $\phi$  and an increase in the  $Y$ . Under saline conditions, Martrigz and Cosgrove (1987) have suggested the  $\phi$  (50% reduction) to be the controlling factor since the  $Y$  was unaffected by salt treatment. Since we were unable to measure elongation

rate over a range of turgor pressure, as achieved for wheat roots by Pritchard *et al.* (1987; 1988), the direct measurement of the Lockhart parameters did not prove possible. The elastic and plastic components of  $\phi$  were measured with tensiometer after 5 and 144 hours of the stress onset (Table 1). Both of the components did not change for any of the NaCl concentrations applied even after 5 hours. A decrease was observed after 144 hours but this was true in case of control plants too, indicating that this decrease might be due to some other reasons such as tissue maturity and not the salt stress. A potential error in the technique that should not be ruled out is the measurement in the whole whorl of leaves in the stem to represent the first leaf extensibility. Our results are similar to the findings of Cosgrove and Sovonick-Dunford (1989) who suggest that for pea stems Instron-extensibility is not a reliable indicator of the wall properties that governs growth as the two do not correlate.

It must be noted that our explanation of wall properties regulating growth is based on the apparent absence of turgor pressure change in expanding cells upon salt stress. In conclusion, it must be stated that it is impossible to exclude regulation by turgor pressure change below our limit of resolution. The data presented here represent the current state-of-the-art in studying growth at single cell resolution. Hopefully future developments in techniques will throw more light on this issue.

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## REFERENCES

- Acevedo, E., T.C. Hsiao and D.W. Henderson. 1971. Immediate and subsequent growth responses of maize leaves to change in water status. *Plant Physiol.* 48: 631-636.
- Arif, H. 1990. Water relations of salt stressed wheat. Ph.D. Thesis, Univ. of Wales, Bangor, U.K.
- Cleland, R.E. 1984. The instron technique as a measure of immediate-past wall extensibility. *Planta*, 160: 514-520.
- Cosgrove, D.J. and S.A. Sovonick-Dunford. 1989. Mechanisms of gibberellin-dependent stem elongation in peas. *Plant Physiol.* 89: 184-191.
- Greenway, H. and R. Munns. 1983. Interaction between growth, uptake of Cl and Na and water relations of plants in saline environments. I. Slightly vacuolated cells. *Plant, Cell and Environ.* 6: 567-574.
- Hoagland, D.R. and D.I. Arnon. 1950. The water culture method for growing plants without soil. *Calif. Agri. Exp. Stn. Cir.* No. 347.
- Hüsken, D., E. Steudle and U. Zimmermann. 1978. Pressure probe technique for measuring water relations in higher plants. *Plant Physiol.* 61: 158-163.
- Kemp, D.R. 1980. The location and size of the extension zone of emerging wheat leaves. *New Phytol.* 84: 729-737.
- Lockhart, J.A. 1965. An analysis of irreversible plant cell elongation. *J. Theo. Biol.* 8: 264-275.
- Margritz, C. and D.J. Cosgrove. 1987. The biophysical mechanism of reduced growth in *Pisum sativum* seedling under saline conditions. p. 145. In: D.J. Cosgrove and D.P. Knievel (ed.). *Physiology of Cell Expansion During Plant Growth*. Am. Soc. Plant Physiol., USA.
- Mastuda, K. and A. Riazi. 1981. Stress-induced osmotic adjustment in growing regions of barley leaves. *Plant Physiol.* 68: 571-576.
- Matthews, M.A., E. Van Volkenburgh and J.S. Boyer. 1984. Acclimation of leaf growth to low water potentials in sunflower. *Plant, Cell and Environ.* 7: 199-206.
- Michelena, V.A. and J.S. Boyer. 1982. Complete turgor maintenance at low water potentials in the elongation region of maize leaves. *Plant Physiol.* 69: 1445-1449.
- Neumann, P.M., E. Van Volkenburgh and R.E. Cleland. 1988. Salinity stress inhibits bean leaf expansion by reducing turgor not wall extensibility. *Plant Physiol.* 88: 233-237.
- Pritchard, J. 1988. The control of growth rate in wheat seedling roots. Ph.D. Thesis, Univ. of Wales Bangor, U.K.
- Pritchard, J., A.D. Tomos and R.G. Wyn Jones. 1987. Control of wheat root elongation growth. I. Effects of ions on growth rate, wall rheology and cell water relation. *J. Exp. Bot.* 38: 948-959.
- Pritchard, J., R.G. Wyn Jones and A.D. Tomos. 1988. Control of wheat root growth. II. The effects of excision on growth, wall rheology and root anatomy. *Planta*, 176: 399-405.
- Rashid, A. 1986. Mechanism of salt tolerance in wheat (*Triticum aestivum* L.). Ph.D. Thesis, Univ. of Agri., Faisalabad, Pakistan.
- Rich, T.C.G. and A.D. Tomos. 1988. Turgor pressure and phototropism in *Sinapis alba* L. seedling. *J. Exp. Bot.* 39: 291-299.
- Thiel, G., J. Lynch and A. Läuchli. 1988. Short term effects of salinity on the turgor and elongation of growing barley leaves. *J. Plant Physiol.* 132: 38-44.
- Thomas, A., A.D. Tomos, J.L. Stoddart, H. Thomas and C.J. Pollock. 1989. Cell expansion rate, temperature and turgor pressure in growing leaves of *Lolium temulentum* L. *New Phytol.* 112: 1-5.
- Tomos, A.D. 1988. Cellular Water Relations of Plants. p. 186-277. In: F. Franks (ed.) *Water Science Review*. 3. Cambridge Univ. Press, Cambridge, U.K.
- Van Volkenburgh, E., S. Hunt and W.J. Davies. 1983. A simple instrument for measuring cell-wall extensibility. *Ann. Bot.* 51: 669-672.